BRAF V600E expression in ameloblastomas-A 36-patient cohort from Helsinki University Hospital

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Abstract

Objectives: We aimed to investigate BRAF V600E percentage immunohistochemically in ameloblastomas of a single institute cohort. We were interested if age, location, histological properties, or tumor recurrence depend on the BRAF status.

Subjects, Materials and Methods: We had 36 formalin-fixed, paraffin-embedded ameloblastoma tissue samples of patients treated at the Helsinki University Hospital between the years 1983-2016. Tissue sections underwent immunohistochemistry by Ventana BenchMark XT immunostainer using Ms Anti-Braf V600E (VE1) MAB. We used R 3.4.2 and RSudio1.1.383 to conduct statistical analysis for BRAF positivity and earlier onset as well as tumor location. We used X^2 -tests and 2 by 2 table functions to determine connections between BRAF positivity and recurrence, growth pattern, and type.

Results: BRAF positive tumors occurred in younger patients compared to BRAF negative tumors (p = 0.015) and they located mostly to the mandible (p = <0.001). Growth patterns were limited to two in BRAF negative tumors when BRAF positive tumors presented with one to four growth patterns (p = 0.02). None of the maxillary tumor showed BRAF positivity and of these, 72.2% recurred.

Conclusions: An immunohistochemical BRAF marker could be a beneficial tool to predict the outcome of patients with this aggressive, easily recurring tumor.

INTRODUCTION

Odontogenic tumors arise from the tooth forming apparatus. These are a highly heterogenic group of rare tumors originating from the cells of epithelial, ectomesenchymal, and, or mesenchymal elements occurring in the oro-maxillo-facial area. Ameloblastomas, clinically the most common odontogenic tumor, arise from the odontogenic epithelium and resembles histologically the dental enamel organ and the dental lamina. Ameloblastomas indicate dental integrity by expressing transcription factors of early dental epithelia, *PITX2, MSX2*, and *DLX1, 2, 3, 4* (Heikinheimo et al., 2015). According to WHO, ameloblastomas are classified as ameloblastoma (intraosseus; solid/multicystic ameloblastoma), unicystic ameloblastoma, and peripheral ameloblastoma. Ameloblastomas grow in follicular, plexiform, acanthomatous, granular cell or basal cell patterns. (El-Naggar, Chan, Grandis, Takata, & Slootweg, 2017). In general, ameloblastomas are benign, locally invasive, and recurring tumors, usually located in the posterior portion of the mandible (80%), affecting people in all age groups (10 to 80 years), equally men and women. Due to the tumor's high growth potential, patients may undergo severely mutilating surgery following difficult prosthetic treatments.

Several studies have addressed the genetic background of ameloblastoma (Brown et al., 2014; Kurppa et al., 2014; Sweeney et al., 2014). Mutation in the mitogen-activated protein kinase (MAPK) pathway gene *BRAF* in which glutamic acid at codon 600 replaces the amino acid valine (V) has been found in 46-66% of solid or multicystic ameloblastomas, mainly in the mandible. The MAPK signaling pathway orchestrates cell proliferation, differentiation, migration, and survival. For example a *BRAF* mutation, commonly found in melanoma, thyroid, and colorectal cancers, leads to a constant activation of these functions (Holderfield, Deuker, McCormick, & McMahon, 2014). Activation of the MAPK pathway via BRAF has been suggested to function also in various odontogenic tumors with ameloblastomatous components, making the BRAFV600E a potential marker for diagnostic purposes (Brown et al., 2014; Brunner, Bihl, Jundt, Baumhoer, & Hoeller, 2015). It has also been suggested that BRAF inhibitors could be a potential treatment modality for ameloblastomas. (Fernandes, Girardi, Bernardes, Fonseca, & Fregnani, 2018; Fregnani et al., 2017). *BRAF* mutated tumors locate more often to the mandible, and occur in younger patients. Ameloblastomas without *BRAF* mutations recure earlier and locate in the maxilla (Brown et al., 2014). Although reports of the genetic background of the development of ameloblastoma have emerged, the

exact mechanisms of cell differentiation, oncogenesis, and progression of this tumor remain unsolved (Nagi, Sahu, & Rakesh, 2016).

Our study aimed to present the BRAF V600E immunohistochemical status in 36 ameloblastomas from patients treated at Helsinki University Hospital (HUH) focusing on the following questions: which is the percentage of BRAF positivity, is age and tumor location a determining factor in BRAF-samples, does recurrence depend on BRAF-mutations, and does BRAF expression differ between histological or growth pattern variants.

MATERIAL AND METHODS

Patient and tissue material

Patients treated at Department of Oral and Maxillofacial Diseases, HUH for ameloblastoma during 1983-2016 were included. There were 36 non-decalcified formalin-fixed, paraffin-embedded whole tissue sample blocks available for examination. The tissue samples came from the archive of Department of Pathology, HUH, information on these specimens from Q-pati registration files, and clinical data from the HUH's patient archives and electronic patient records. The Finnish National Supervisory Authority for Welfare and Health (Valvira) granted permission for the use of patient samples. The Ethics Committee of Surgery and HUH's Internal Review Board approved the study protocol (Dnro 151/13/03/02/2015).

We have reported the clinical data and demographics of this cohort in detail in our previous publication (Kelppe et al., 2018). Of the 34 cases of our previous study, we had to discard four due to lack of tissue samples or because of tissue decalcification preventing the use of immunohistochemistry. To enlarge the cohort, six cases were added: four patients treated for a recurred tumor (primary tumor treated elsewhere) and two cases that were discarded from the previous study because of insufficient clinical data but having tissue available for this study, giving a total of 36 patients.

Immunohistochemistry

Formalin-fixed, paraffin-embedded, 3µm thick tissue sections underwent automated immunohistochemistry by Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, AZ, USA) using Ms Anti-Braf V600E (VE1) Mab with a 32 minutes incubation time, Spring Bioscience diluted to 1:1500 and visualized by OptiView DAB IHCv3(Ventana) with amplification. The specimens were counterstained with haematoxylin. Melanoma tissue served as a positive control. In the negative control tissue the primary antibody was left out.

Scoring of BRAF immunohistochemistry

An oral pathologist (JK) examined the slides and considered cytoplasmic immunoreactivity as positive when present regardless of staining intensity (Fig.1.) The positivity was present only in tumoral tissue. Normal tissue was negative.

Statistical analysis

We calculated risk ratios for BRAF positive tumors and an earlier onset age as well as for BRAF positive tumors and location. We used X^2 -tests, and where relevant, 2 by 2 table functions to determine connections between BRAF positivity and recurrence, BRAF positivity and growth patterns, and BRAF positivity and ameloblastoma types. A p -value equal or less than 0.05 was considered significant. We used a logistic regression model with BRAF as an outcome variable to determine the odds ratio and the confidence interval for the location adjusted for gender and age. We conducted the analyses using R 3.4.2 (R Core Team 2017) and RStudio 1.1.383.

RESULTS

Table 1. presents patient information. The male to female ratio was 1.25:1, men having an average age of 55.9 (range 13-83) and women 45.8 (range 18-71). Recurrence occurred (or the tumor was a recurrence to begin with) in 14 (38.9%) cases, 8 female and 6 male. Figure 1. presents recurrences occurring in maxillary and mandibular tumors.

BRAF status

BRAF positivity was found only in tumors located to the mandible (n = 26/36) (Table 1.). The average age of patients having a BRAF positive tumor was 46.8 and of those with a BRAF negative tumor 65.2.

Mandible

Of the studied ameloblastoma cases, 29 (80.6%) located to the mandible, of which 26 (89.7%) were BRAF positive. Only 9 (31%) mandibular tumors recurred, all of them being BRAF positive. None of the mandibular BRAF negative tumors showed recurrence, though the amount of BRAF negative tumors was low (n = 3) (Table 1.).

Maxilla

Ameloblastoma occurred in the maxilla in 7 (19.4%) cases, all BRAF negative. Of maxillary tumors 5 (71.4%) recurred, 4 in male and 1 in a female patient (Table 1.).

Histology

Of ameloblastomas, 27 (75%) were solid/multicystic, 7 (19.4%) unicystic and 2 (5.5%) of peripheral type. In solid/muticystic ameloblastomas BRAF positivity occurred two times more often (18/27) than BRAF negativity (9/27). All unicystic ameloblastomas were BRAF positive. The over all mixture of growth patterns was versitile. Maxillary tumor seems to present more simple growth patter variations. BRAF positive tumors showed more variance in growth patterns (Figure 2.). Acanthomatosis was present in almost half (17/36) of all samples but BRAF negative tumors did not show acanthomatosis. Figure 3. presents examples of histology of BRAF positive and BRAF negative tumors. In BRAF positive tumors, the positivity seemed to vanish when desmoplasia was present (Figure 4.)

Statistics

A relation between age and BRAF positivity was seen (p = 0.015) inclining that BRAF positive tumors occur earlier in life. In addition, a correlation between BRAF positive tumors and a mandibular location was seen (p = <0.001). None of the BRAF negative tumors had more than two growth patterns (p = 0.02). Nevertheless, recurrence or ameloblastoma type did not seem to be dependent on the BRAF status (Table 2.). In a logistic regression model, with BRAF+/- as outcome variable, adjusted for age and gender, odds ratio for location was 2.32 (95% CI 1.77-3.05) indicating BRAF positive tumors to locate in the mandible.

DISCUSSION

Here we report BRAFV600E immunopositivity in our ameloblastoma material of 36 tumors. Previous studies show BRAF-mutation in more than 62.7% of ameloblastomas (Brown & Betz, 2015; Brown et al., 2014; Diniz et al., 2015; do Canto et al., 2019; Kurppa et al., 2014; Sweeney et al., 2014). Our results are in line with and further extend these findings, the corresponding proportion being 72.2% (26/36). Our cohort demonstrates male predominance in BRAF negative tumors, most of them located to the maxilla. Additionally, BRAF negative patients had an 18.4 years higher average age than the BRAF positive patients. Maxillary tumors of which all were BRAF negative, showed recurrence in 71.4% (5/7), while all recurring tumors in the mandible were BRAF positive (45%, 9/20). The overall recurrence rate, regardless of location, reached up to 38.9% (14/36). BRAF negative tumors demonstrated a simpler histologic scenery than the BRAF positive tumors.

BRAF status is considered a predictive and prognostic tool for determining the course of ameloblastoma patients (Brown & Betz, 2015; Brown et al., 2014; Fregnani et al., 2017). BRAF inhibitors have even been used in treating ameloblastoma patients (Fernandes et al., 2018). Distinct anatomical distribution between ameloblastomas carrying different alterations in SHH and MAPK pathways are rather indisputable: *SMO* mutations exists mainly in maxillary tumors and *BRAF* mutations in mandibular tumors (Brown et al., 2014; Sweeney et al., 2014). Our results are in line with these findings since all BRAF positive tumors were located to the mandible. Additionally, patients with a BRAF negative tumor were older than patients having a BRAF positive tumor as shown in previous works as well (Brown et al., 2014). Brown et al. reported a mean age difference between patients with a BRAF negative

and BRAF positive tumors of 22.7 years (Brown & Betz, 2015), corresponding figure of the present study was 18.4 years. When regarding the overall average age of our patients, one must take into account the four cases of already recurred tumors increasing the average age. Although our material is limited, it seems that male predominance is present in maxillary, more often in recurring tumors. Instead in mandibular tumors the male to female ratio equal 1:1.07, recurrence having female predominance (7/9 female and 2/9 male).

BRAF positivity seemed to vary between different single growth patterns even though no assosciations came forth. In addition we found, that within a single tumor, the BRAF positivity seemed to diminish in desmoplastic areas. The reason for this finding remains unclear. Desmoplastic and plexiform ameloblastomas are the least likely tumors to recure (Hong et al., 2007). In this cohort, there was no solely desmoplastic tumors. Plexiform growth pattern often appeared with other growth patterns. Follicular, acanthomatous, and granular cell growth patterns were recurring most often in a study by Hong et al (2007). In our study, most tumors that recurred had plexiform and or follicular growth pattern. In our study tumors with only follicular growth pattern were BRAF positive. None of the tumors with three or more different growth patterns within one tumor was BRAF negative. Acanthomatosis occurred in 47.2 % of all ameloblastomas and all acanthomatous tumors were BRAF positive. The reason for this can only be speculated. Both recurring and non-recurring tumors presented with all histological growth patterns. In addition, all unicystic ameloblastomas were BRAF positive being in line with research done by Heikinheimo et. al in which they speculate unicystic ameloblastoma and ameloblastoma possibly being a part of the spectrum of the same disease (Heikinheimo et al., 2019).

It has been discussed that immunohistochemistry for identifying BRAFV600E mutated protein from formalin-fixed, paraffin-embedded samples is reliable compared to molecular techniques, thus making it a beneficial tool to detect these mutations as part of the normal diagnostic (Brown et al., 2014; Capper et al., 2011). Recently also opposite results have emerged (Szymonek et al., 2017). Decalcification with formic acid however affects immunoreactivity. When preparing a tumor sample for normal diagnostic examination, these aspects should be taken into consideration.

A larger patient cohort could have provided a more reliable statistical analysis. In addition, some samples were over 30 years old which might affect the staining intensity. On the other hand, it was surprising how well immunohistochemistry functioned on older samples. In

some cases, biopsies or other suboptimal samples were the only usable tissue which makes growth pattern examination hard and less reliable.

To conclude: our study confirms the previous results on BRAF positivity compared with location and age of ameloblastoma patients. The clinical use of BRAF immunohistochemistry applied on a representable tissue sample, could give beneficial information in diagnosis and surgical management.

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References

Brown, N. A., & Betz, B. L. (2015). Ameloblastoma: A review of recent molecular pathogenetic discoveries. *Biomark Cancer, 7s2*, 19-24. doi:10.4137/BIC.S29329

Brown, N. A., Rolland, D., McHugh, J. B., Weigelin, H. C., Zhao, L., Lim, M. S., . . . Betz, B. L. (2014). Activating *FGFR2–RAS–BRAF* mutations in ameloblastoma. *Clinical Cancer Research, 20*(21), 5517-5526. doi:10.1158/1078-0432.CCR-14-1069

Brunner, P., Bihl, M., Jundt, G., Baumhoer, D., & Hoeller, S. (2015). BRAF p.V600E mutations are not unique to ameloblastoma and are shared by other odontogenic tumors with ameloblastic morphology. *Oral Oncology, 51*(10), e77-e78. doi:10.1016/j.oraloncology.2015.07.010

Capper, D., Preusser, M., Habel, A., Sahm, F., Ackermann, U., Schindler, G., . . . von Deimling, A. (2011). Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathologica, 122*(1), 11-19. doi:10.1007/s00401-011-0841-z

Diniz, M. G., Gomes, C. C., GuimarÃ£es, B.,Viana Antonini, Castro, W. H., Lacerda, J., Cardoso, S. V., . . . Gomez, R. S. (2015). Assessment of BRAFV600E and SMOF412E mutations in epithelial odontogenic tumours. *Tumor Biology, 36*(7), 5649-5653. doi:10.1007/s13277-015-3238-0

do Canto, A. M., da Silva Marcelino, Barbara,Michaela Reis, Schussel, J. L., Wastner, B. F., Sassi, L. M., CorrÃªa, L., . . . Braz-Silva, P. (2019). Immunohistochemical analysis of BRAF V600E mutation in ameloblastomas. *Clinical Oral Investigations, 23*(2), 779-784. doi:10.1007/s00784-018-2494-y

El-Naggar, A. K., Chan, J. K. C., Grandis, J. R., Takata, T., & Slootweg, P. J. (Eds.). (2017). *WHO classification of head and neck tumours* (4th ed.). Lyon: WHO/IARC.

Fernandes, G. S., Girardi, D. M., Bernardes, J. P. G., Fonseca, F. P., & Fregnani, E. R. (2018). Clinical benefit and radiological response with BRAF inhibitor in a patient with recurrent ameloblastoma harboring V600E mutation. *BMC Cancer, 18*(1), 887; 887-887. doi:10.1186/s12885-018-4802-y

Fregnani, E. R., Perez, D. E. d. C., Almeida, O. P. d., Fonseca, F. P., Soares, F. A., Castroâ€ • Junior, G., & Alves, F. (2017). BRAFâ€ • V600E expression correlates with ameloblastoma aggressiveness. *Histopathology, 70*(3), 473-484. doi:10.1111/his.13095

Heikinheimo, K., Huhtala, J. -., Thiel, A., Kurppa, K. J., Heikinheimo, H., Kovac, M., . . . Morgan, P. R. (2019). The mutational profile of unicystic ameloblastoma. *J Dent Res, 98*(1), 54-60. doi:10.1177/0022034518798810

Heikinheimo, K., Kurppa, K. J., Laiho, A., Peltonen, S., Berdal, A., Bouattour, A., . . . Morgan, P. R. (2015). Early dental epithelial transcription factors distinguish ameloblastoma from keratocystic odontogenic tumor. *J Dent Res, 94*(1), 101-111. doi:10.1177/0022034514556815

Holderfield, M., Deuker, M. M., McCormick, F., & McMahon, M. (2014). Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. *Nature Reviews.Cancer, 14*(7), 455-467. doi:10.1038/nrc3760

Hong, J., Yun, P. -., Chung, I. -., Myoung, H., Suh, J. -., Seo, B. -., . . . Choung, P. -. (2007). *Long-term follow up on recurrence of 305 ameloblastoma cases* (Apr;36(4) ed.). 283-8: International Journal of Oral and Maxillofacial Surgery. doi:org/10.1016/j.ijom.2006.11.003

Kelppe, J., Hagström, J., Sorsa, T., Suominen, A. L., Apajalahti, S., Haglund, C., & Thorén, H. (2018). Ameloblastoma: A retrospective single institute study of 34 subjects. *Acta Odontologica Scandinavica,* , 1-6. doi:10.1080/00016357.2018.1532530

Kurppa, K. J., Catón, J., Morgan, P. R., Ristimäki, A., Ruhin, B., Kellokoski, J., . . . Heikinheimo, K. (2014). High frequency of BRAF V600E mutations in ameloblastoma. *The Journal of Pathology, 232*(5), 492-498. doi:10.1002/path.4317

Nagi, R., Sahu, S., & Rakesh, N. (2016). Molecular and genetic aspects in the etiopathogenesis of ameloblastoma: An update. *J Oral Maxillofac Pathol,* (3), 497. doi:10.4103/0973-029X.190954

Sweeney, R. T., McClary, A. C., Myers, B. R., Biscocho, J., Neahring, L., Kwei, K. A., . . . West, R. B. (2014). Identification of recurrent SMO and BRAF mutations in ameloblastomas. *Nature Genetics, 46*(7), 722-725. doi:10.1038/ng.2986

Szymonek, M., Kowalik, A., Kopczyński, J., Gąsior-Perczak, D., Pałyga, I., Walczyk, A., . . . Kowalska, A. (2017). *Immunohistochemistry cannot replace DNA analysis for evaluation of BRAF V600E mutations in papillary thyroid carcinoma* (Aug 24;8(43) ed.). 74897-74909: - Impact Journals LLC. doi:- 10.18632/oncotarget.20451

TABLES

Table 1. Tumor BRAF-expression distribution between mandibula and maxilla and between male and female patients. Average age of patients with BRAF positive and BRAF negative is also shown. The total ameloblastoma count was 36.

		BRAF+	**BRAF-**
Mandibula	**29/36 (80,6%)**	**26/29 (89,7%)**	**3/29 (10,3%)**
Male	14/29(48,3%)	12/29 (41,4%)	2/29 (6,9%)
Female	15/29(51,7%)	14/29 (48,3%)	1/29 (3,4%)
Recurrence	9/29 (31%)	9/29 (31%)	0/29 (0%)
No recurrence	20/29 (69%)	17/29 (58,6%)	3/29 (10,3%)
Maxilla	**7 /36(19,4%)**	**0/7(0%)**	**7/7(100%)**
Male	6/7 (85,7%)	0	6/7 (85,7%)
Female	1/7 (14,3%)	0	1/7 (14,3%)
Recurrence	5/7 (71,4%)	0	5/7 (71,4%)
No recurrence	2/7 (28,6)	0	2/7 (28,6)
Age (avarage, years)	**51,9**	**46,8**	**65,2**

Table 2. 2 by 2 tables for BRAF and median age, recurrance and location.

		BRAF+	BRAF-	Total	X^2 test; *p*-value
Median age	**Lower age group**	17	2	19	X^2=5.969, *p*-value: 0.015
	Higher age group	9	8	17	
		26	10	36	
Recurrence	**Yes**	17	5	22	X^2=0.21758, *p*-value: 0.6409
	No	9	5	14	
		26	10	36	
Location	**Mandibula**	26	3	29	X^2=22.593, *p*-value: <0.001
	Maxilla	0	7	7	
		26	10	36	

Figure 1. Mandibula-maxillary distribution among male and female patients regarding recurrence. Almost every other mandibular tumor in female patients recurred. In male patients with maxillary tumors every other tumor recurred.

Figure 2. presenting different histologic growth pattern variations in BRAF positive (orange) and BRAF negative (blue) samples. BRAF negative tumors lacked purely follicular growth patterns, never showed more than two growth patterns and never showed acanthomatous metaplasia.

Figure 3. BRAF positivity shown in mandibular tumors. Mandibular tumors (a,e) and the HE staining of the same tumors (b,f). BRAF negativity in maxillary tumors (c,g) and HE staining of these cases (d,h). (Magnification x100)

Figure 4. Follicular or plexiform patterns demonstrate BRAF positivity (a-d). Where desmoplasia is present the tumor seems to lose its round shape and BRAF positivity diminishes. In a x40 magnification little dots of positivity is never the less observed.